"The expert in anything was once a beginner"

CSIR NET – Life Science

Unit 1: Biochemistry

17 ETC and Oxidative Phosphorylation

Points to be covered in this Lecture

- Biological Oxidation
- Redox Potential
- Electron Transport Chain
- Energy Transducer – ATP synthase
- Oxidative Phosphorylation
- Shuttles

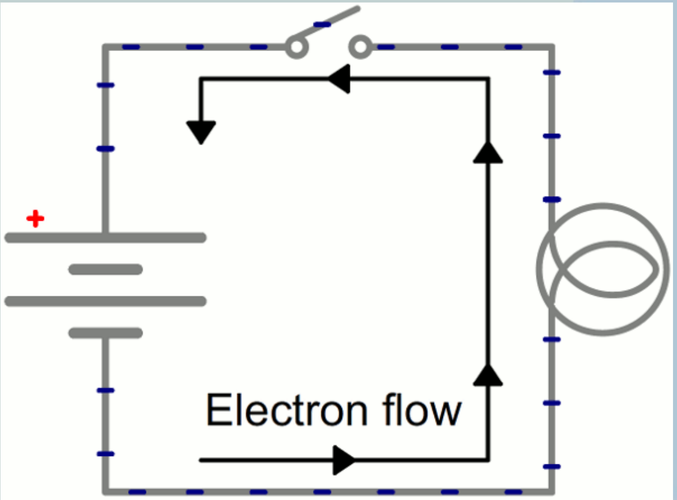
BIOLOGICAL OXIDATION

- Involves the loss of electrons
- Gain of oxygen or the loss of hydrogen.

$\boxed{A} \xrightarrow{e^-} B$

Oxidation — Reductant (Reducing Agent)

Reduction — (oxidizing Agent)



The Flow of Electrons (emf) Can Do Biological Work

Four types of electron transfer occur in cells

1. Directly as electrons:

$$Fe^{2+} \longrightarrow Fe^{3+} + e^-$$

$$Cu^{+} \longrightarrow Cu^{2+} + e^-$$

2. As hydrogen atoms:

$$\boxed{H} \longrightarrow 1H^+ + \boxed{1e^-}$$

$$FADH_2 \longrightarrow FAD + 2H^+ + 2e^-$$

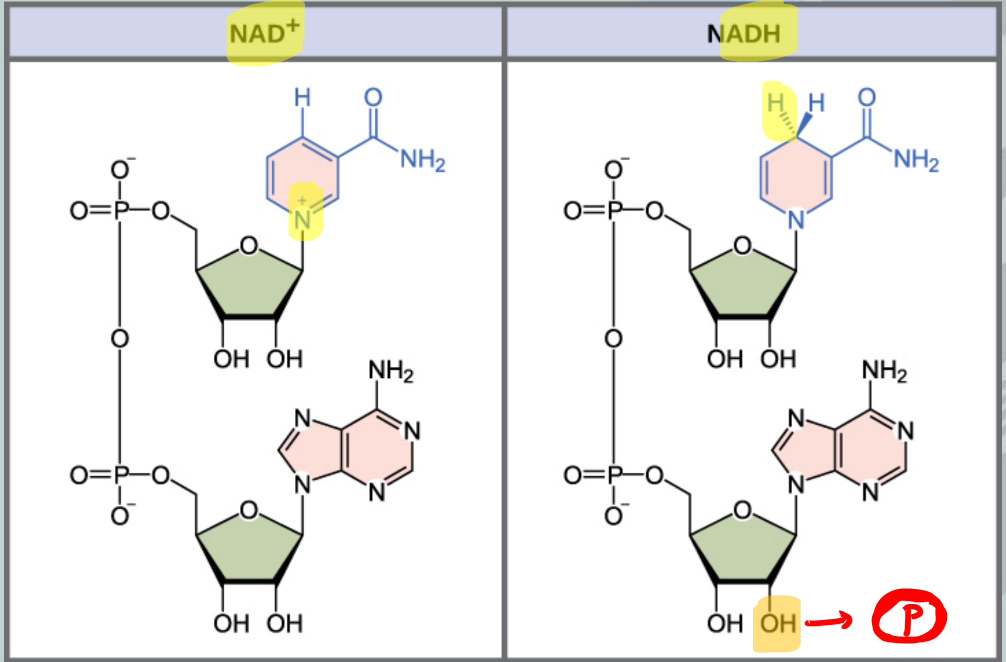
$$FMNH_2 \longrightarrow FMN + 2H^+ + 2e^-$$

3. As a hydride ion (:H⁺) which has two electrons:

$\ddot{H} = 1H^+ + 2e^- = H^-$

$NADH \longrightarrow NAD^+ + 1H^+ + 2e^-$

$NADPH \longrightarrow NADP^+ + 1H^+ + 2e^-$



4. Through direct combination with oxygen:

$$R-H \longrightarrow R-OH$$

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-H \longrightarrow R-\overset{\overset{\displaystyle O}{\|}}{C}-OH$$

Reduction potential (measure in volts)

- Measure affinity for electrons
- Tendency of molecule to gain electrons

$$\downarrow 2H^+ \underset{}{\overset{+2e}{\rightleftharpoons}} H_2 \uparrow \quad \boxed{0\ V}$$

$$\uparrow NAD^+ \underset{+1H^+}{\overset{+2e^-}{\rightleftharpoons}} NADH \downarrow$$

1 M — Electron acceptor

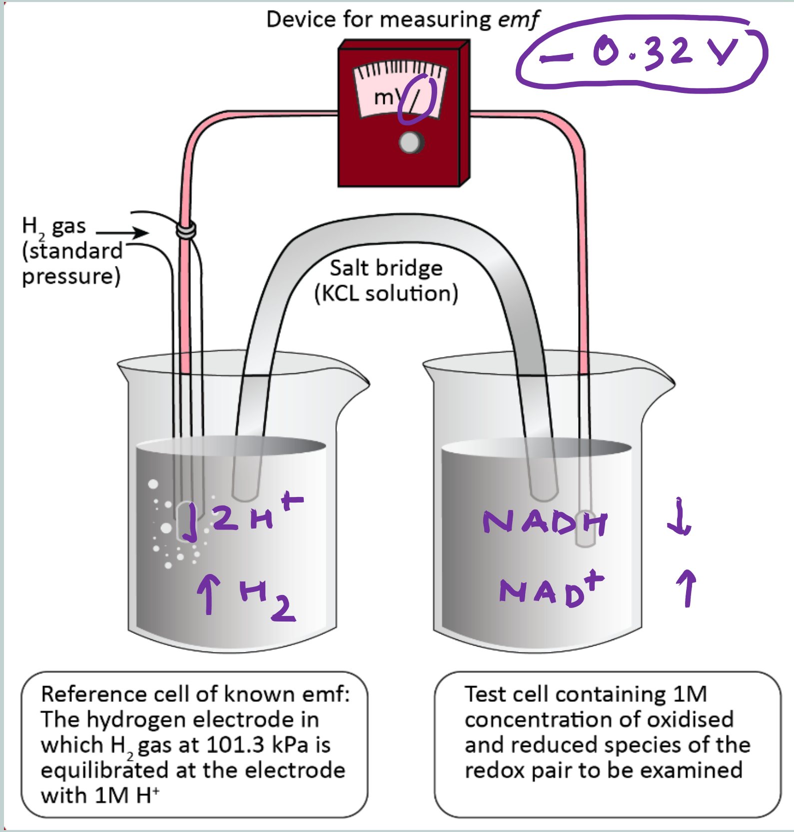
1 M — Electron Donor

The standard reduction potential, E^0

- Reduction potential of a substance under standard conditions
- 1 M concentration, 1 atmospheric pressure, and 25°C., pH = 0 → E^0

  pH = 7 → $E^{0'}$
- Measured relative to the standard hydrogen electrode (0 volts)

Standard reference



$$H^+ + e^- \rightarrow \frac{1}{2} H_2$$

Test cell containing 1M (Oxidised/Reduced species)

$$NADH \longrightarrow NAD^+ + 2e^- + H^+$$

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- $\longrightarrow$ $Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + e^- $\longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- $\longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + e^- $\longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^-$ $\longrightarrow$ ubiquinol + H_2	0.045
Fumarate^{2-} + $2H^+ + 2e^-$ $\longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^-$ $\longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate^{2-} + $2H^+ + 2e^-$ $\longrightarrow$ malate^{2-}	−0.166
Pyruvate$^-$ + $2H^+ + 2e^-$ $\longrightarrow$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ $\longrightarrow$ ethanol	−0.197
FAD + $2H^+ + 2e^-$ $\longrightarrow$ $FADH_2$	−0.219*
Glutathione + $2H^+ + 2e^-$ $\longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ $\longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ $\longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate + CO_2 + $2H^+ + 2e^-$ $\longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- $\longrightarrow$ ferredoxin (Fe^{2+})	−0.432

$\frac{H_2O}{O_2}$ +0.8

$\frac{FADH_2}{FAD}$ −0.22

$\frac{NADH}{NAD^+}$ −0.32

$\frac{NADPH}{NAD^+}$ −0.324

Electron flow will be low redox potential to high redox potential

Standard reduction potentials can be used to calculate the free-energy change

$\Delta E^\circ (E^\circ_B - E^\circ_A)$

	A		B	
E°	-0.32	$\longrightarrow$	-0.22	$(-0.22) - (-0.32) = +0.1$
E°	-0.3	$\longrightarrow$	0	$(0) - (-0.3) = +0.3$
E°	-0.3	$\longrightarrow$	$+0.2$	$(0.2) - (-0.3) = +0.5$
E°	$+0.2$	$\longrightarrow$	$+0.3$	$(0.3) - (0.2) = +0.1$
	Low		High	

Spontaneous = ΔE° = positive change

$\boxed{\Delta G^\circ = \text{negative}}$

$$\Delta G^\circ = -n F \Delta E^\circ$$

$$= -n \times 23 \times \Delta E^\circ \ (\text{K.cal})$$

$$= -n \times 96.5 \times \Delta E^\circ \ (\text{KJ/mole})$$

n = number of electron

Fe^{2+} or Cu^+ = $1e^-$

FAD, NAD^+, NADP, UQ = $2e^-$

F= Faraday constant (96.5 kJ/V. mol or 23 [illegible]) Kcal/V.mol

Consider the reaction in which acetaldehyde is reduced by the biological electron carrier NADH:

Electron Acceptor Electron donar

Acetaldehyde + NADH + H^+ → ethanol + NAD^+

Reduction oxd

The relevant half-reactions and their $E^{0'}$ values are:

(1) Acetaldehyde + $2H^+$ + $2e^-$ → ethanol $E^{0'}$= - 0.2 V

(2) NAD + $2H^+$ + $2e^-$ → NADH + H^+ $E^{0'}$= - 0.3 V

Calculate $\Delta G^{0'}$ *(Given F = 96.5 kJ/V.mol)*?

$$\Delta G^0 = -nF\Delta E^0$$
$$= -2 \times 96.5 \times \Delta E^0$$
$$= -2 \times 96.5 \times 0.1$$
$$= -193 \times 0.1$$
$$\boxed{\Delta G^0 = -19.3 \text{ KJ/mole}}$$

→ standard condition

$$\Delta E^0 = (E^0_{Acceptor}) - (E^0_{Donar})$$
$$= (-0.2V) - (-0.3)$$
$$= -0.2 + 0.3$$
$$= +0.1$$

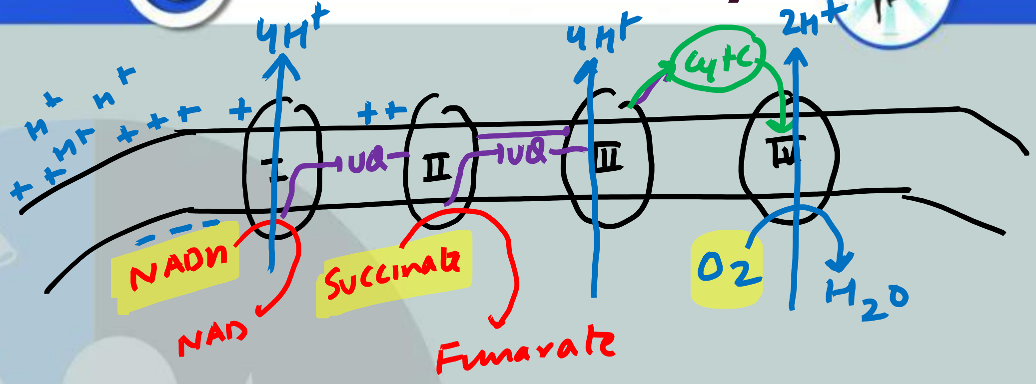
Nerst Equation:

- Cellular condition
- Physiological condition

$$E = E^0 + \frac{0.26}{n} \ln \frac{(Electron\ Acceptor)}{(Electron\ Donor)}$$

$$\Delta G = -nF\Delta E$$

$$\Delta E = E_{Acceptor} - E_{Donor}$$



Electron Transport Chain

Location:

- Inner membrane of mitochondria
- Plasma membrane of bacteria

Components:

Four immobile complexes → I, II, III, IV (UQ, cytc)

Ubiquinone (lipid soluble) and Cytochrome C (protein) are mobile

Functions

Oxidize NADH or Succinate

Transfer electron to oxygen

Generate electrochemical gradient

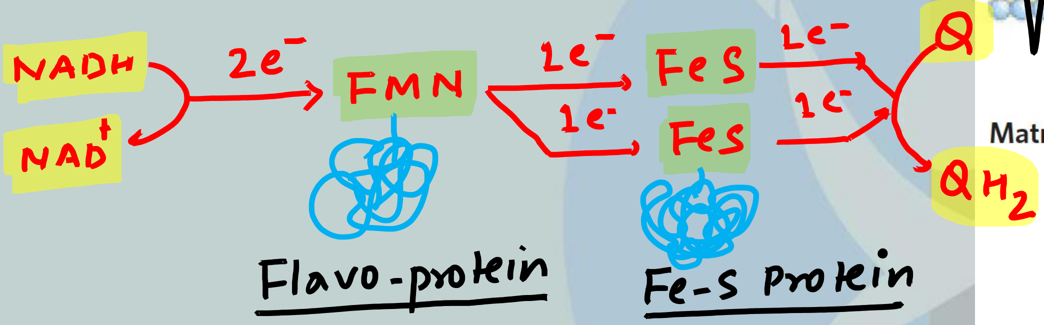
Electrical Gradient : outside will be (+)

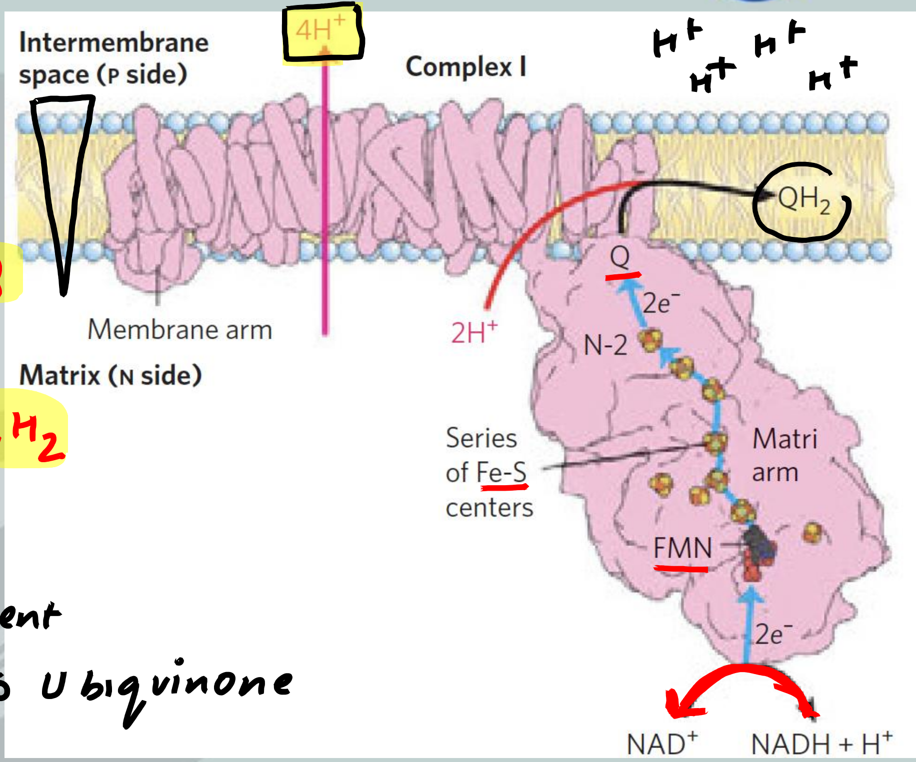
Chemical Gradient : outside more H^+ conc^n

NADH-Q reductase (Complex I):

NADH Dehydrogenase complex

- One molecule of flavin mononucleotide
- Eight or nine iron–sulfur clusters



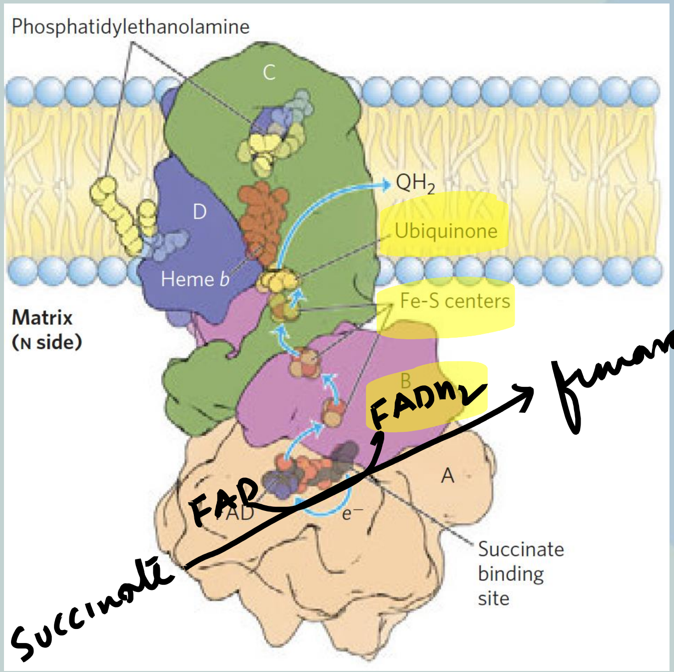


- $4H^+$ are pumped against gradient during transfer $2e^-$ from NADH to Ubiquinone

Complex II: Succinate dehydrogenase complex

✓ Succinate to FAD to electron-transferring flavoprotein (ETF) → Fes → Quinone

Near heme b or the quinone-binding site: Anti-oxidant → Prevents ROS



Succinate → $FADH_2$ → ETF → FeS → Q / QH_2

Fumarate

Also has heme b → Antioxidant

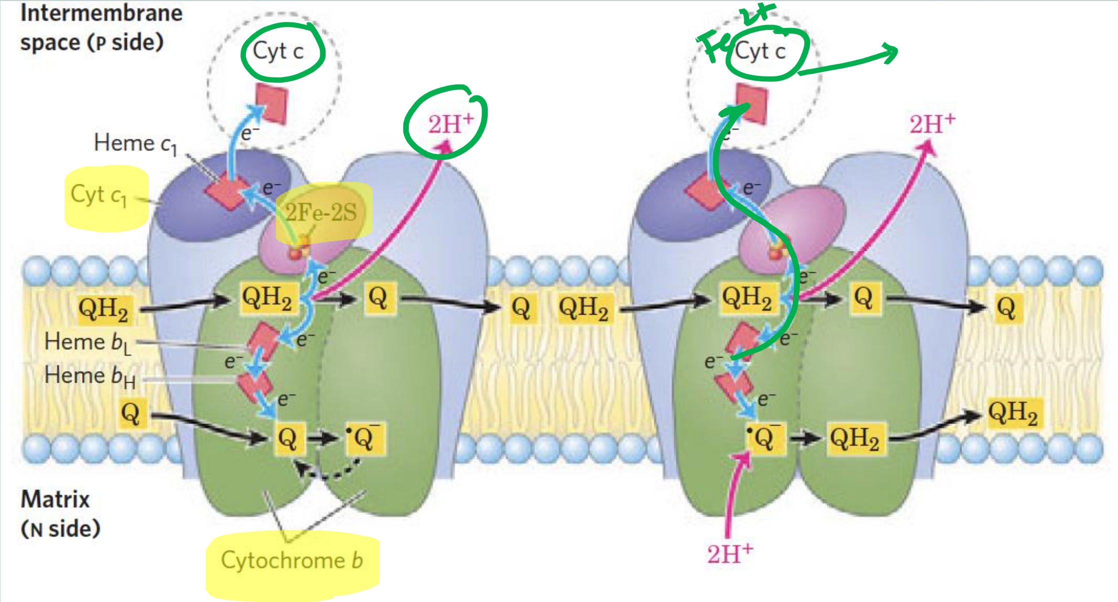
Ubi-Quinone

Lipid soluble

- Ubiquinone (CoQ_{10}): Ten C5 isoprenoid units (50 carbon terpene — Polyterpene)
- Transfer electron between complex $\boxed{\text{I and III}}$ or $\boxed{\text{II and III}}$
- Protects cells from oxidative damage by neutralizing free radicals

$$\underset{\text{oxidized}}{UQ} \xrightarrow[1H^+]{1e^-} \underset{\text{Semi-ubiquinone}}{U\dot{Q}H} \xrightarrow[1H^+]{1e^-} \underset{\text{Reduced ubiquinone}}{U\ddot{Q}H_2}$$

Q-cytochrome c reductase (Complex III): Cyt b - c_1 complex



$QH_2 + Q + \text{cyt } c_1\text{(oxidized)} \longrightarrow Q + {}^{\cdot}Q^- + 2H^+_P + \text{cyt } c_1\text{(reduced)}$

$QH_2 + {}^{\cdot}Q^- + 2H^+_N + \text{cyt } c_1\text{(oxidized)} \longrightarrow Q + 2H^+_P + QH_2 + \text{cyt } c_1\text{(reduced)}$

Net equation: $QH_2 + 2\text{ cyt } c_1\text{(oxidized)} + 2H^+_N \longrightarrow Q + 2\text{ cyt } c_1\text{(reduced)} + 4H^+_P$

Haeme protein

- Consists of cytochrome b cytochrome c1, and the Rieske iron-sulfur protein.
- Pumping protons across the inner mitochondrial membrane

  Q cycle = $4H^+$ are pumped
- Can result in the leakage of electrons to oxygen, forming superoxide

$\boxed{QH_2} \xrightarrow{1e^-} Fes \xrightarrow{1e^-} cyt\ c_1 \xrightarrow{1e^-} cyt\ c$

$QH_2 \xrightarrow{1e^-} cyt\ b_L \xrightarrow{1e} cyt\ b_H \xrightarrow{1e^-} QH_2$

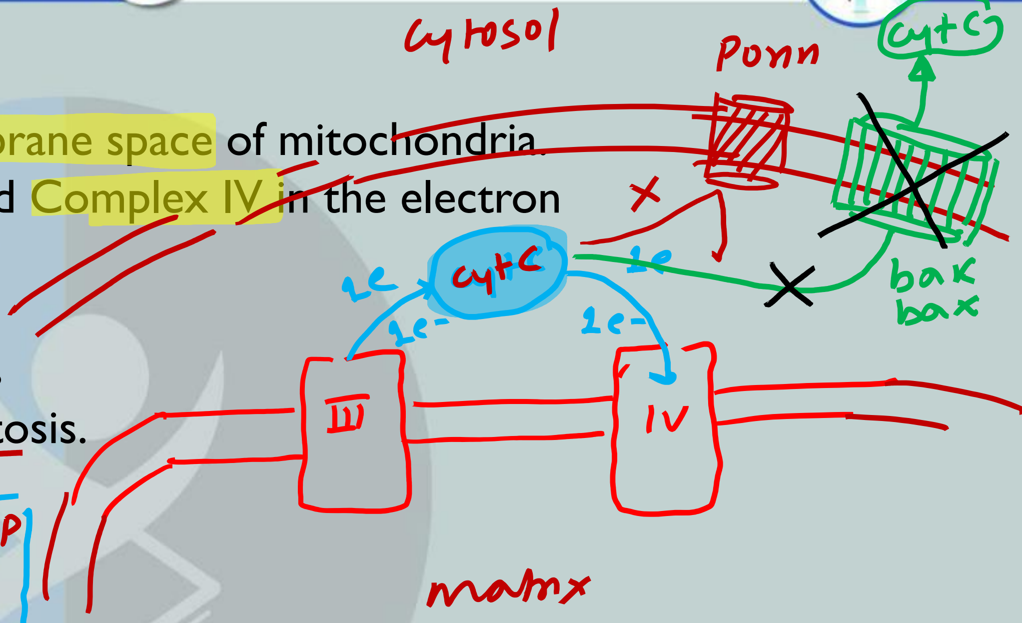
Q-cycle

Cytochrome c

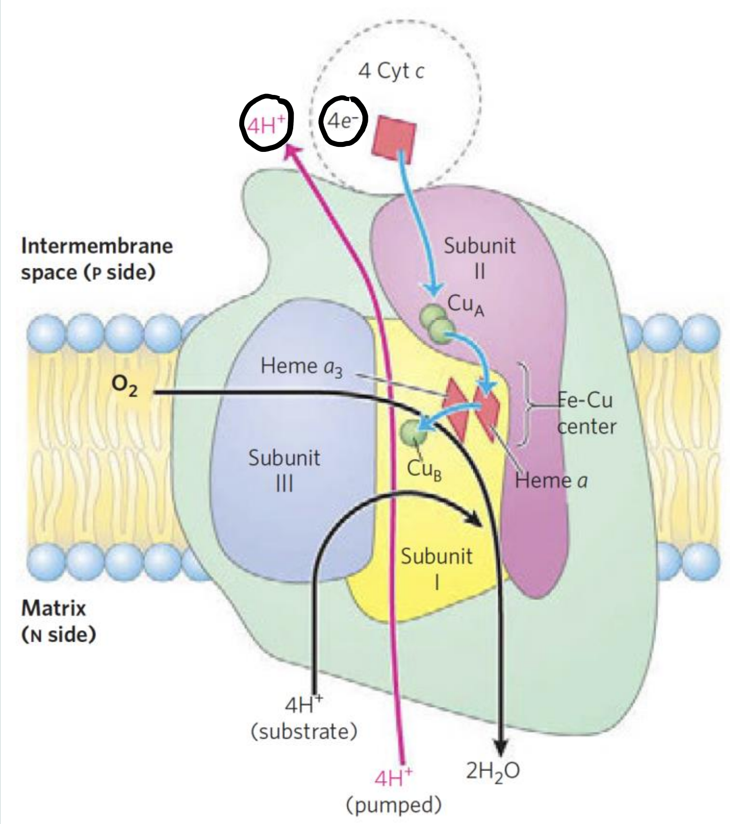
- Soluble protein located in the intermembrane space of mitochondria.
- Electron shuttle between Complex III and Complex IV in the electron transport chain.
- It contains a heme group
- Highly conserved across different species
- Involved in the intrinsic pathway of apoptosis.

cytosol → cyt c + Apaf-1 + ATP + procaspase-9

Apoptosome complex



Cytochrome c oxidase (Complex IV):



- Transfer of electrons from cytochrome c to molecular oxygen
- Translocates protons across the inner mitochondrial membrane $2e^- \rightarrow 2H^+$ $\quad 4e^- \rightarrow 4H^+$
- Contains two heme groups (Cyt a and a3) and two copper centers (CuA and CuB).

cyt c

cyt a e^- — CuA e^-

cyt a_3 e^- — Cu B e^-

$4e^-$

O_2 $\rightarrow$ $2H_2O$

$4H^+$

metabolic water

Electron transport chain inhibitors:

Rotenone
Amytal
Barbiturates
Piercidin

⊖ Complex-I

Malonate
Carboxin

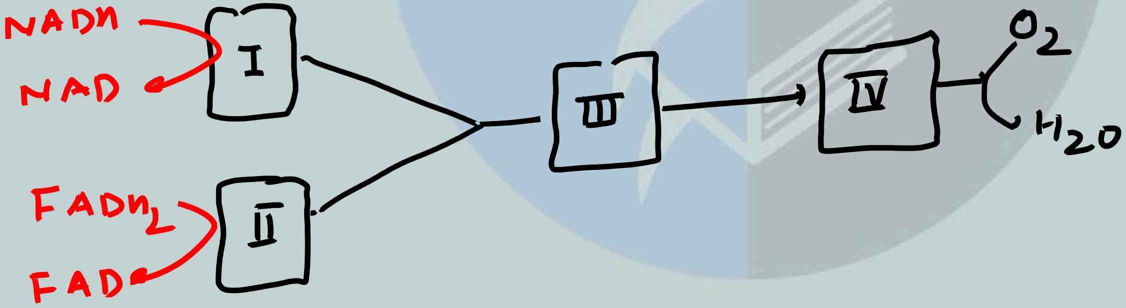
⊖ Complex II

Phenformin
Dimercaprol
Antimycin A
BAL (British Anti Lewisite)

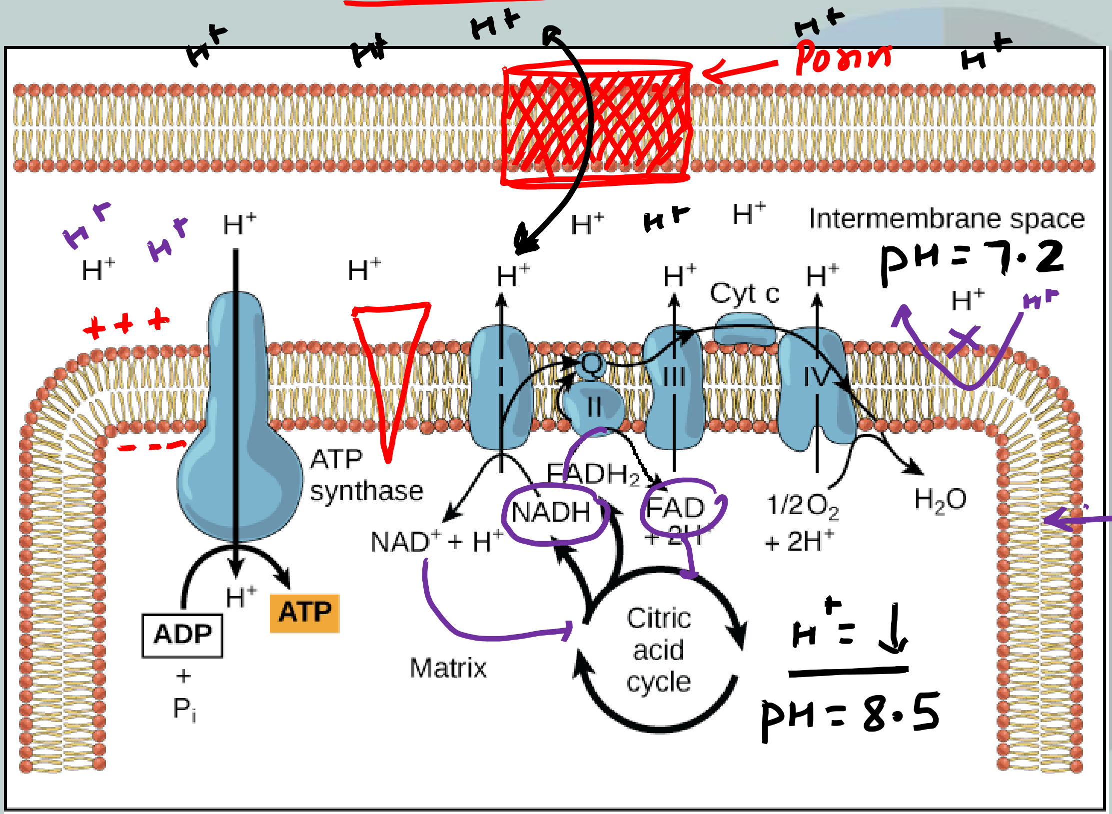
⊖ Complex III

Cyanide
Azide
CO
H_2S

⊖ Complex-IV



Formation of electrochemical (H^+) gradient

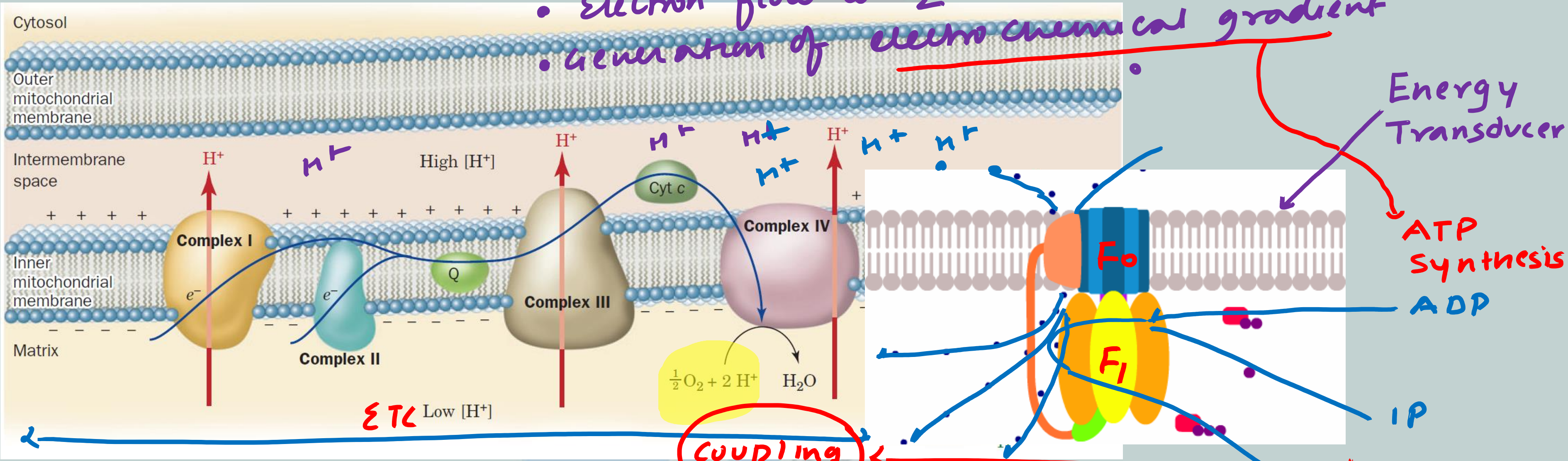


- cytosol or intermembrane space has around 20 times more H^+ as compare to mitochondrial matrix

Inner membrane is immpereable to H^+

Oxidative phosphorylation:

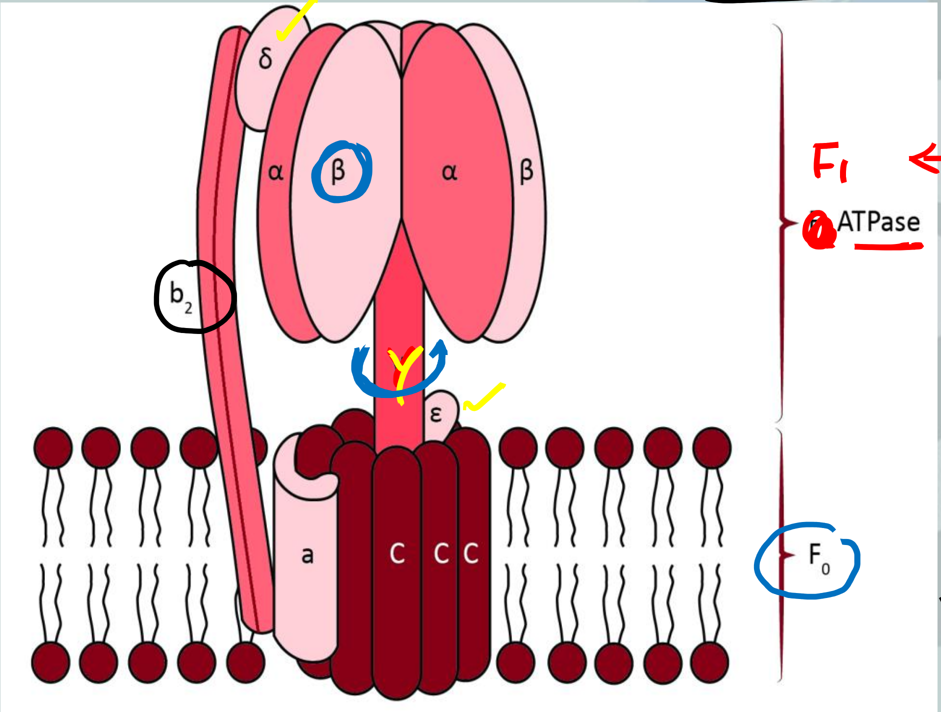
- Oxidation of biomolecule in TCA, β-oxidation
- Production of $FADH_2$ & NADH
- $FADH_2$ & NADH are oxidized by complex I or II
- Electron flow to O_2
- Generation of electrochemical gradient



- Proton motive force
- Rotational catalysis — F_1
- Phosphorylation of ADP to produce ATP

ATP Synthase Is Driven by the Flow of Protons

The F1 Component Has Pseudo-Threefold Symmetry
The Fo Component Includes a Transmembrane Ring



F₁ ← Peripheral protein

$\boxed{3\alpha}$, $\boxed{3\beta}$ → catalytic

1 γ, 1 δ, 1 ε

1a, 2b
10 c - subunit
↓
Fo channel

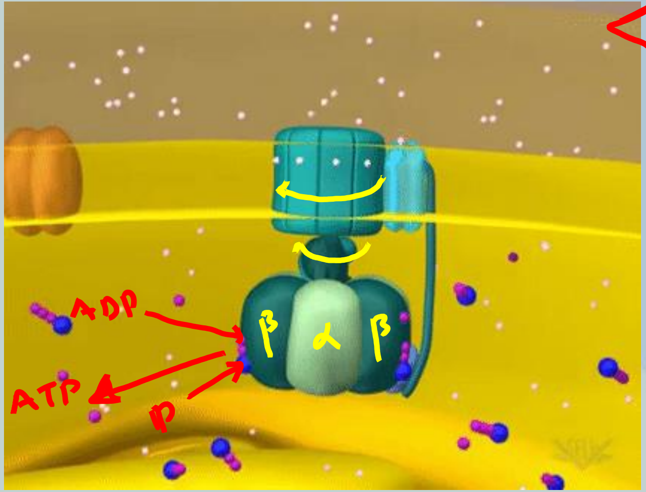
ATP Is Synthesized by the Binding Change Mechanism.

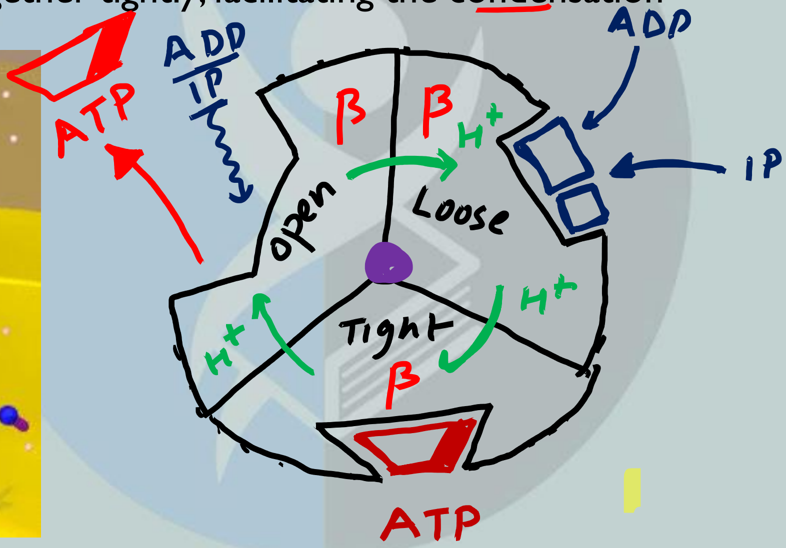
- Each of the β (nucleotide binding sites) three subunits cycle through 3 different conformations due to rotation of Y subunit:

(O)pen: little affinity; ADP and Pi are free to enter (ATP is free to leave) the catalytic site

(L)oose: higher affinity; lose binding of ADP and Pi

(T)ight: Packing the ADP and Pi together tightly; facilitating the condensation

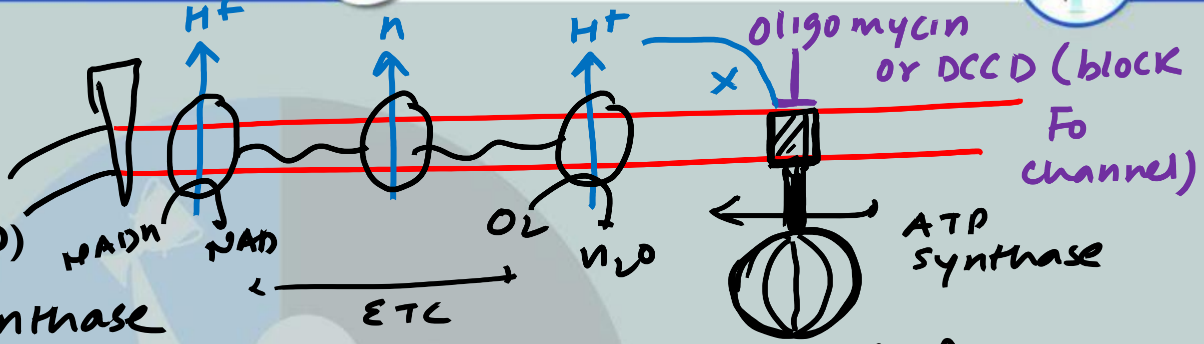




$3H^+$ are needed for 1 ATP synthesis by $F_o - F_1$ particle (ATP Synthase) (F-Type of ATPase)

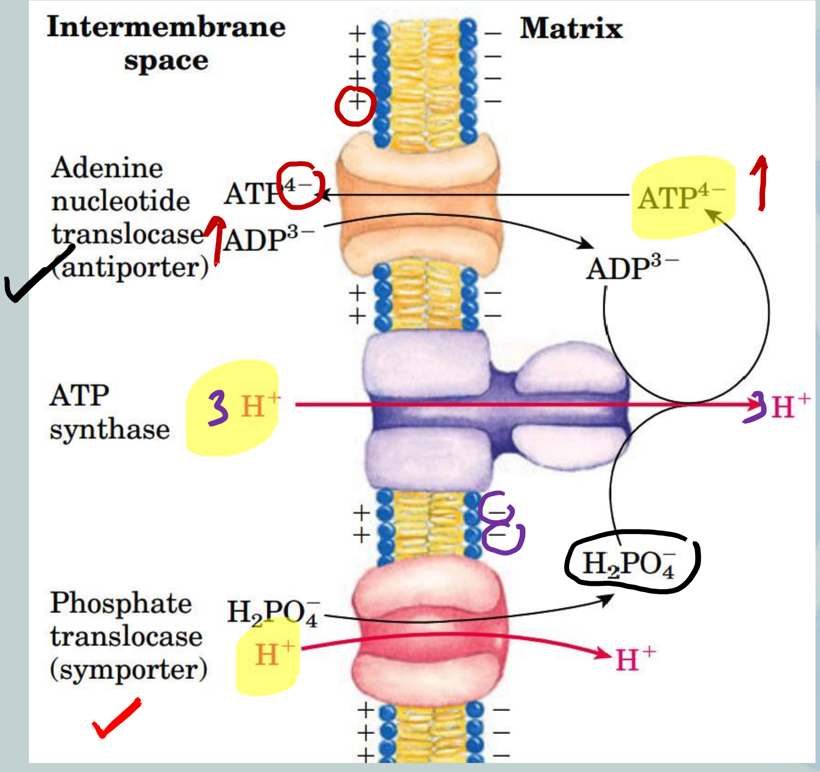
ATP synthase Inhibitors

- Oligomycin
- Dicyclohexylcarbodiimide (DCCD)



Directly inhibit ATP synthase but since electron transport chain is coupled with ATP synthesis thus ETC will also pause.

ADP/ATP transport systems



- ADP/ATP Translocator

- 1 ADP (−3) is exchanged for 1 ATP (−4)
- No energy investment
- Along electrochemical gradient movement

Phosphate Translocator

- movement of phosphate is along chemical gradient but opposite to electrical gradient
- Co-transport : H^+ / phosphate

Energy of H^+ gradient is used for active transport of iP

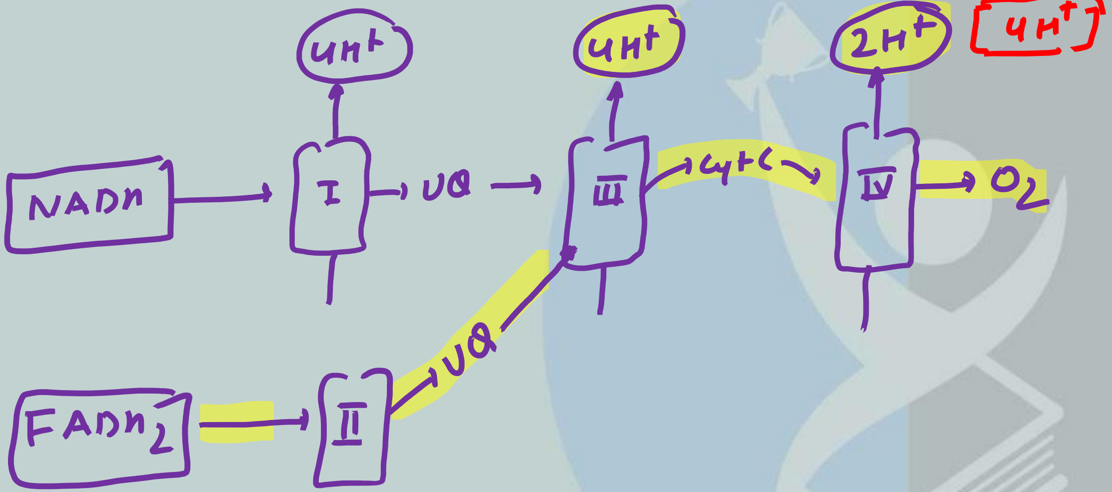
$4H^+ \longrightarrow 1\ ATP$

Atractyloside and bongkrekic acid/ bongkrekate inhibit ATP/ADP translocator

The free energy from NADH to O_2 can drive the synthesis of approximately 2.5 ATP

$4H^+ = 1\ ATP$

The free energy from FADH2 to O_2 can drive the synthesis of approximately 1.5 ATP



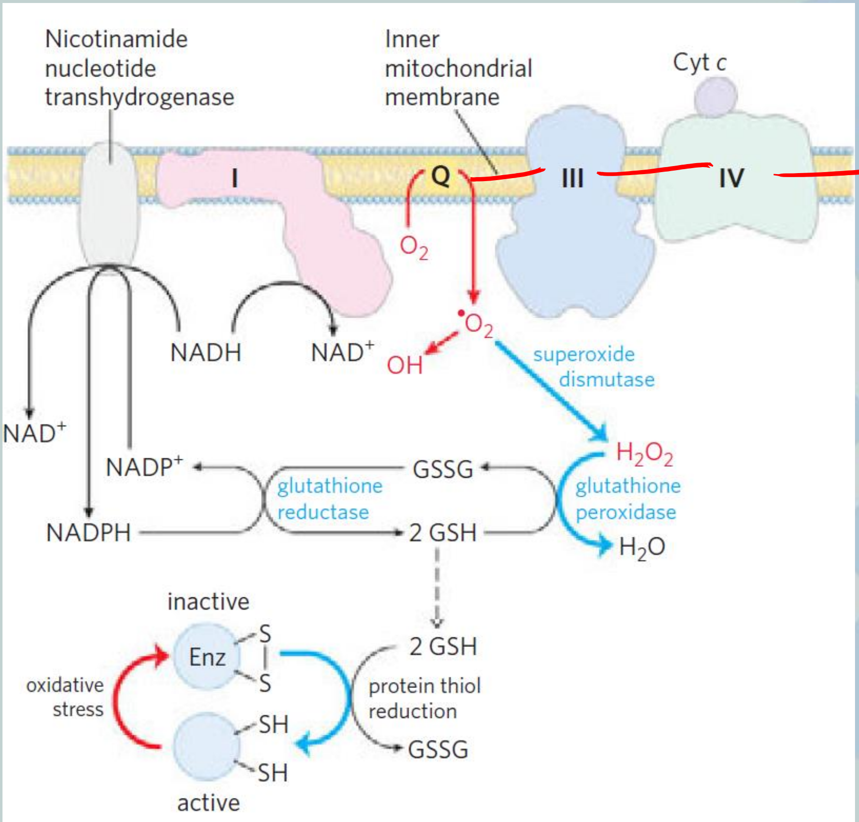
$10H^+$

$6H^+$

$$\frac{10H^+}{4H^+} = 2.5\ ATP$$

$$\frac{6H^+}{4H^+} = 1.5\ ATP$$

Reactive Oxygen Species Are Generated during Oxidative Phosphorylation



- Anti-oxidant machinery
- Neutralize ROS

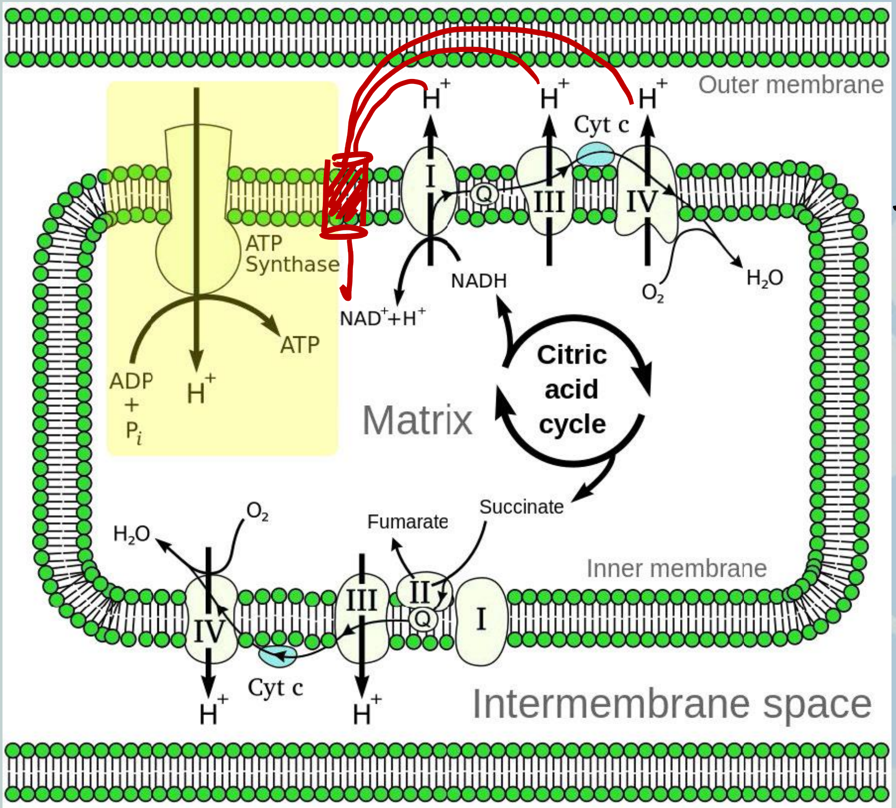
Super oxide Dismutase (Mn^{2+})

$$O_2^- \rightarrow \boxed{H_2O_2}$$

Glutathione Peroxidase (Se)

$$H_2O_2 \rightarrow H_2O + O_2$$

Coupling of ETC with ATP synthesis:

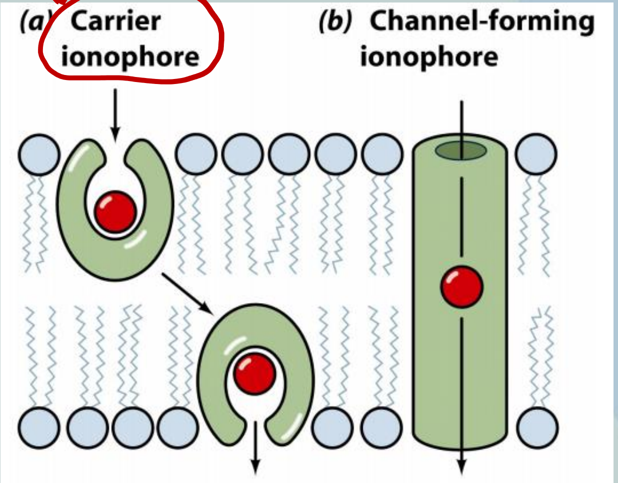


- ETC can function only if H^+ enters back to matrix through Fo channel of ATP synthase.
- Inner membr is immpereable to H^+.

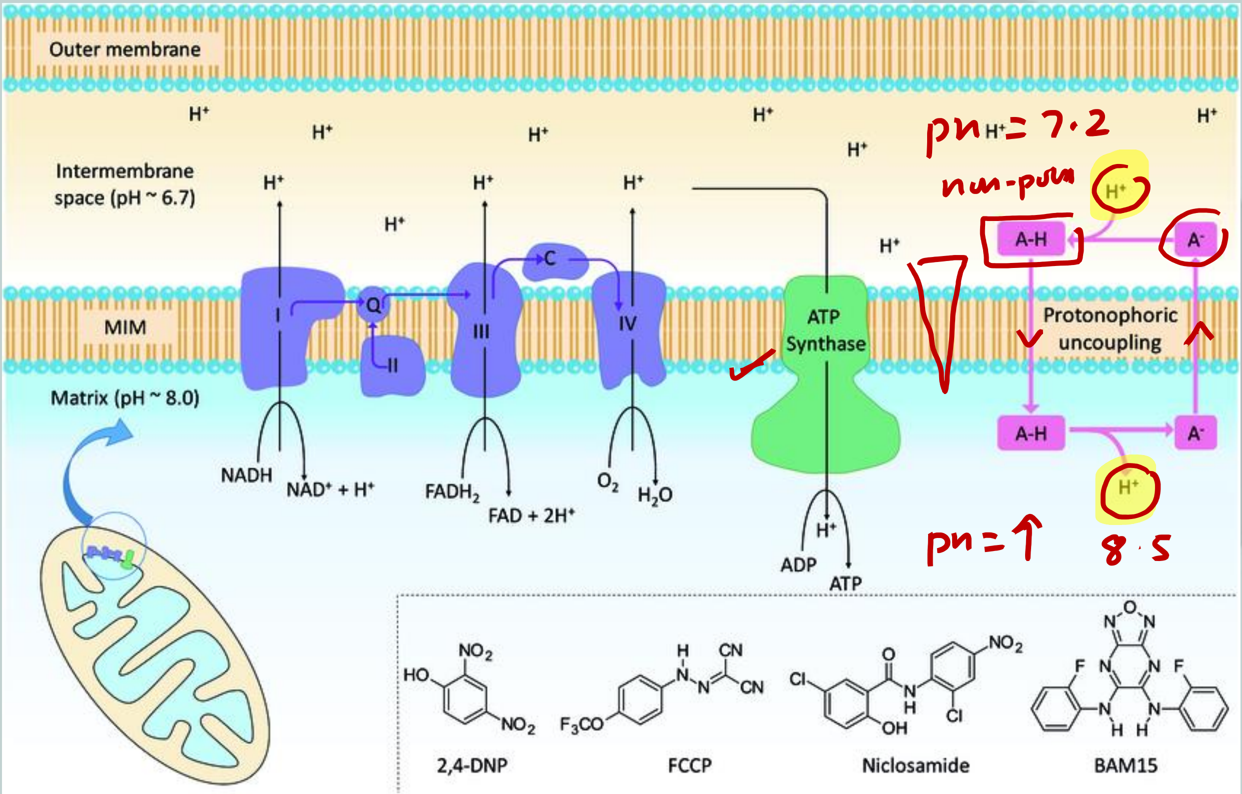
Uncouplers

- Allow electron transport chain to operate in absence of ATP synthesis
- Provide alternative route for entry of H^+ to mitochondrial matrix
- Energy is released in form of heat

1. Ion carrier
2. Ionophore — channel forming



Synthetic uncouplers (Ion carriers):



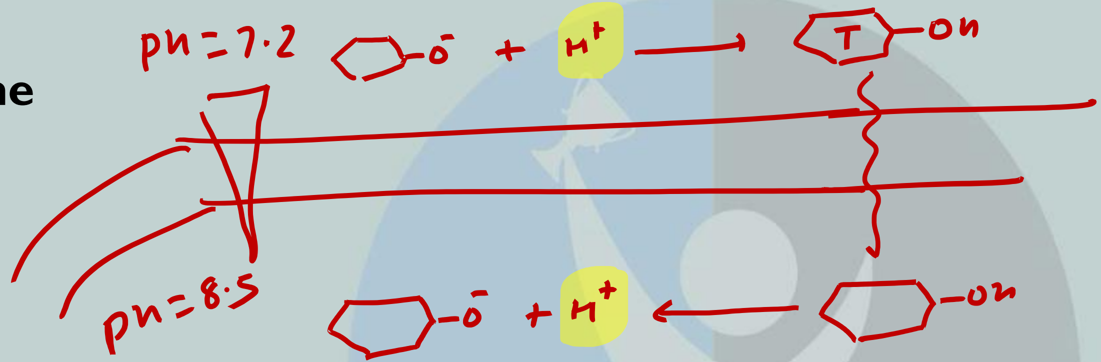
2,4-dinitrophenol:
proton carrier

→ FCCP
→ Aspirin
→ Niclosamide

Physiological uncouplers (ion carriers)

Thyroxine

Bilirubin

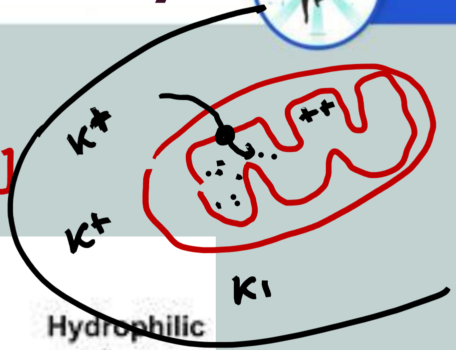


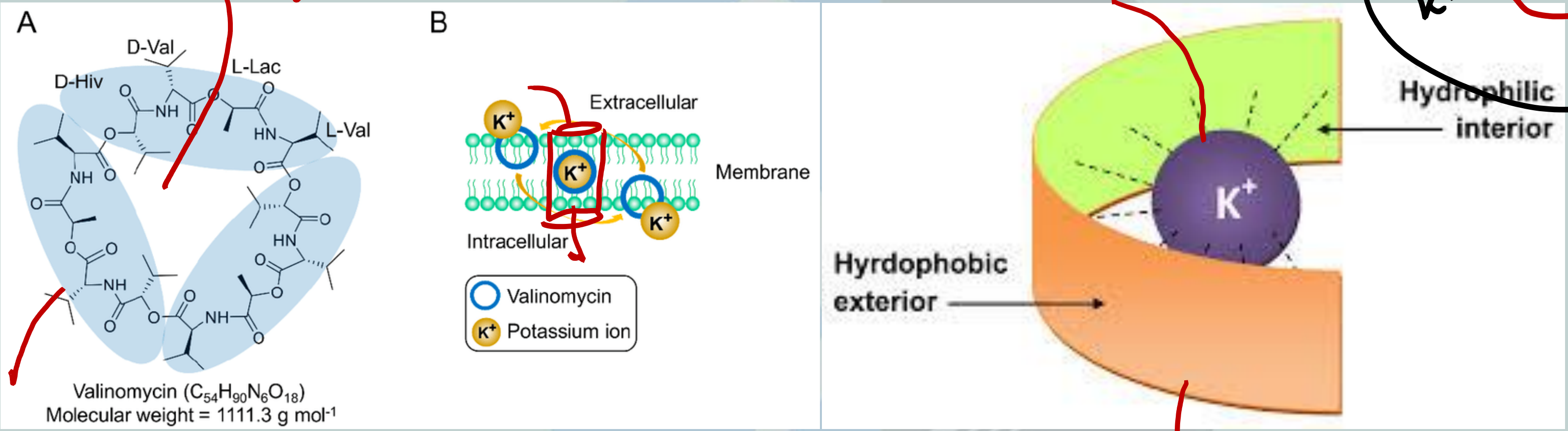
ATP generation = NO
Heat generation = Yes
ETC operate = Yes
O_2 consumption = Yes.

Ionophores

D & L- amino acid
Amphipathic pore forming helix

Antibiotics **valinomycin** and **nigercin** act as **ionophores for** K^+ ion





Valinomycin ($C_{54}H_{90}N_6O_{18}$)
Molecular weight = 1111.3 g mol^{-1}

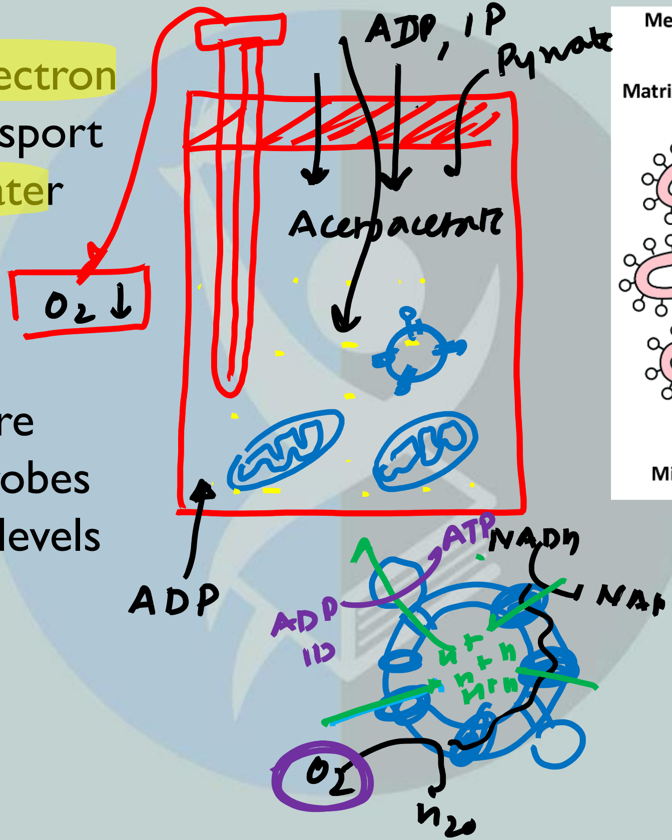
Brown adipose tissue: In polar Animals

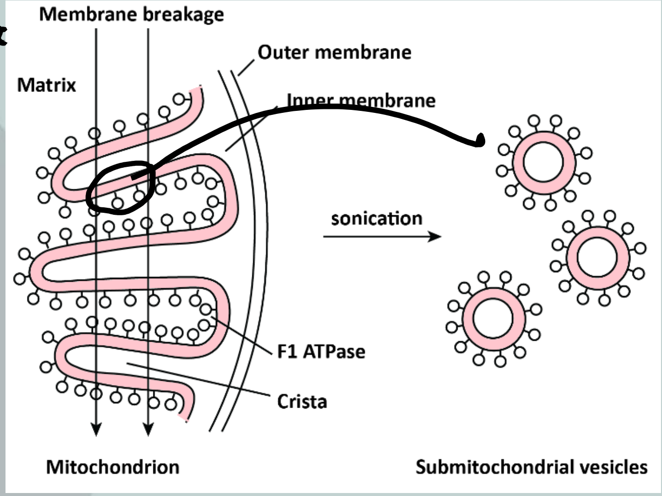
Thermogenin (or uncoupling protein). : Ionophore for H^+ ions

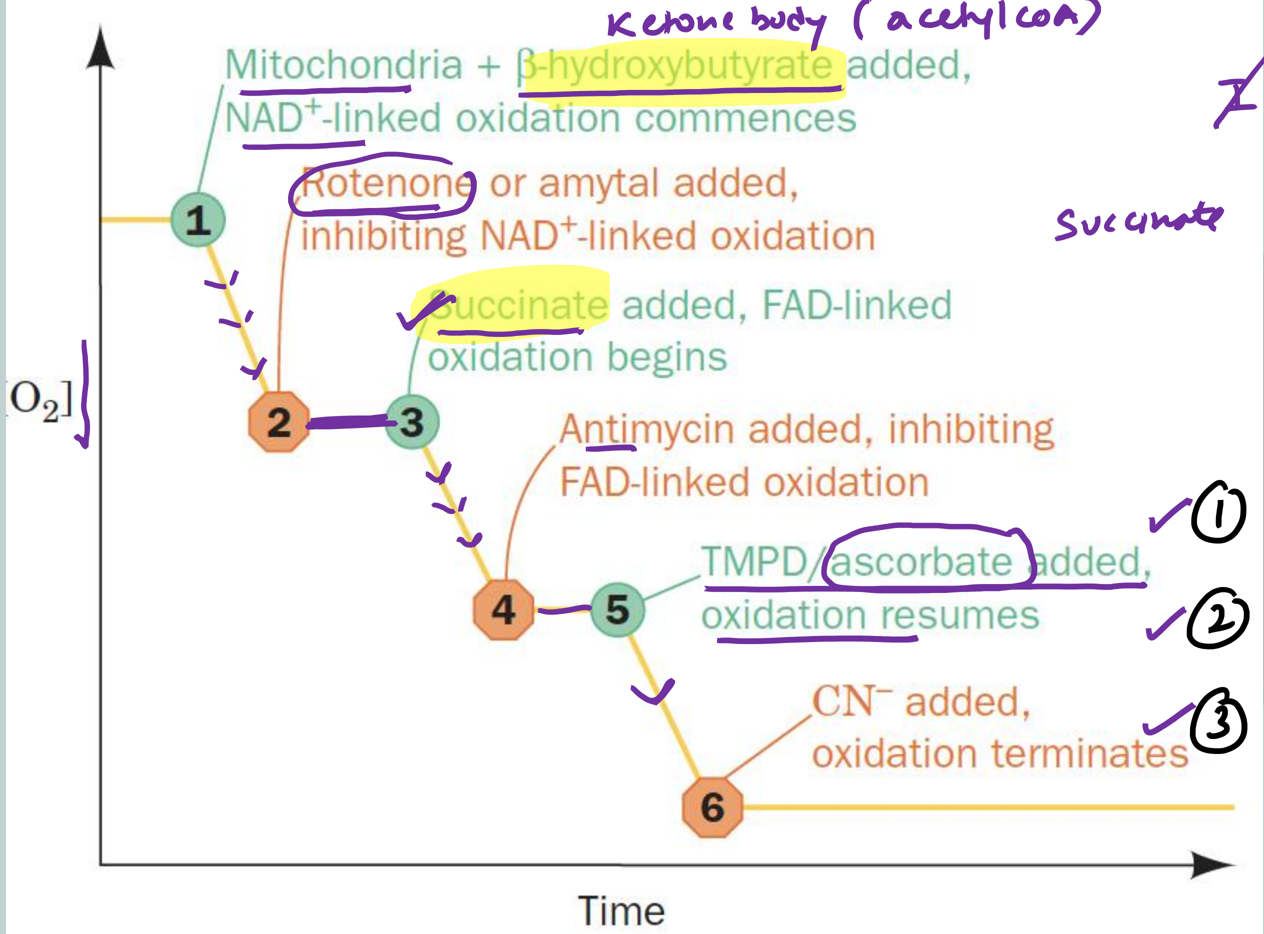
Study of effect of various molecules on electron transport chain

Oxygen serves as the final electron acceptor in the electron transport chain, and its reduction to water marks the completion of the electron transport process.

Clark-type electrodes or more advanced oxygen-sensitive probes are used to measure oxygen levels in a closed system.







Succinate

I ⊣ Rotenone (−)

cyanide

II → III → IV → O2

Antimycin

Vit C
Ascorbate

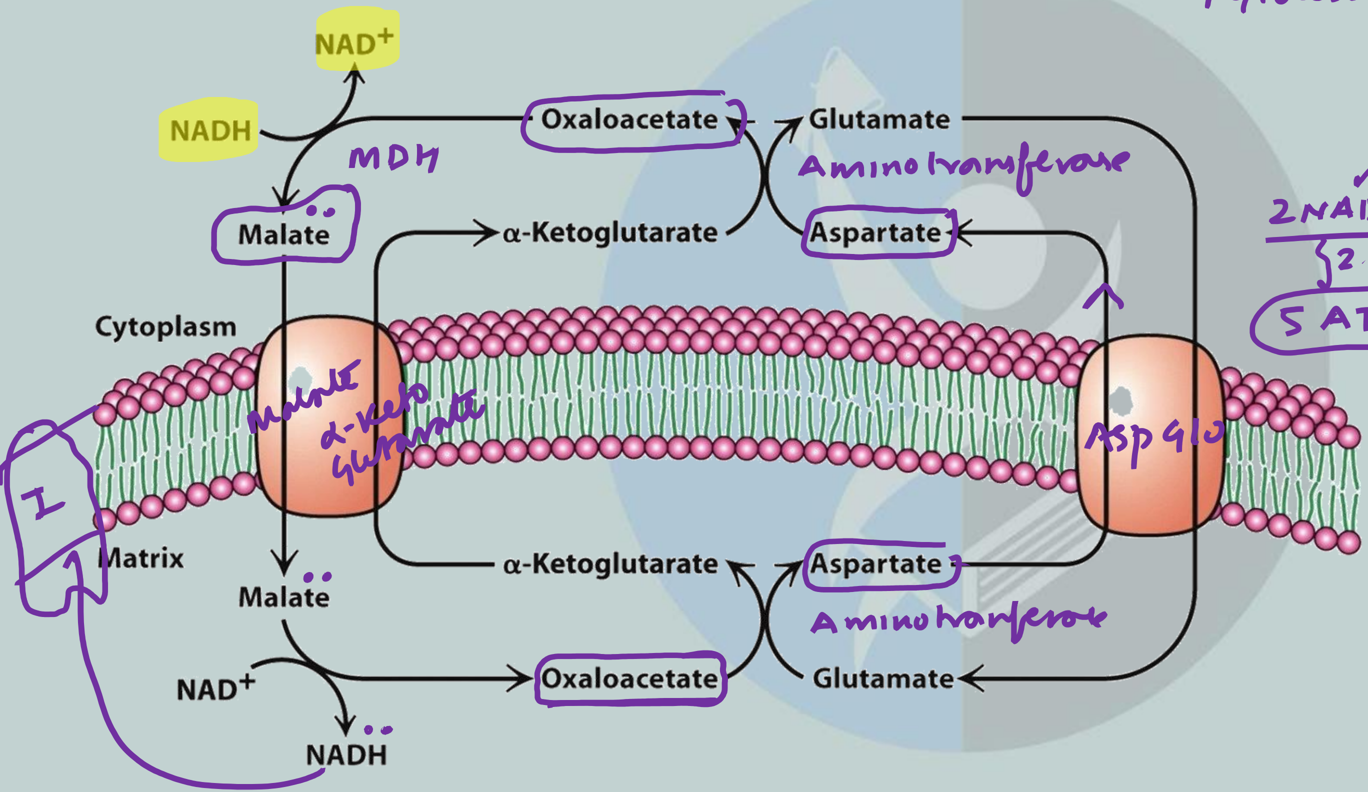
① Rotenone off, Succinate ON → Inhibit complex I

② Antimycin off, Ascorbic acid ON → Inhibit complex III

③ Oligomycin off, 2,4 DNP ON → Inhibit ATP synthase

M A

Malate-Aspartate Shuttle (Heart, liver and kidney):



Glycolysis

1 glucose → 2 pyruvate

2 ATP

2 NADH cytosol

?

2 NADH

2.5

5 ATP

ETC Mitochondria Inner membr

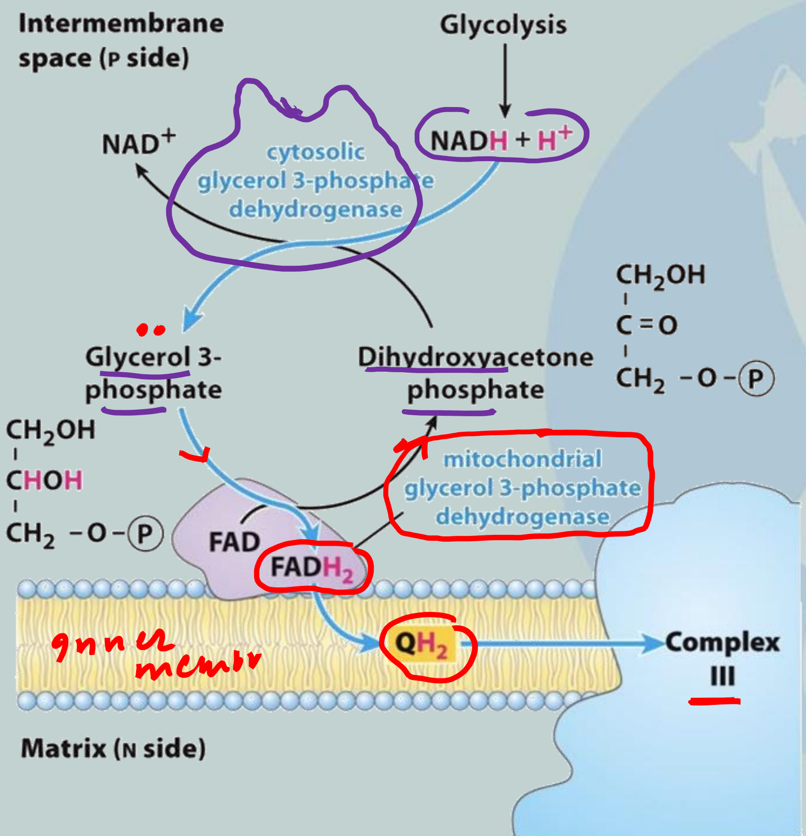
1 glucose

$\boxed{\text{Glycolysis} = 7\ ATP}$

$\boxed{2\ \text{Pyruvate} = 25\ ATP}$

32 ATP

The glycerophosphate shuttle (brain and muscles); Plants



cytosolic glycerol-3-P DH: NADH → NAD$^+$

mitochondrial glycerol-3-P DH: FAD → $FADH_2$

2 NADH ⟶ 2 $FADH_2$ → 1.5 ATP → 3 ATP

1 Glucose

- Glycolysis = 2 + 3 = 5 ATP
- 2 Pyruvate = 25 ATP

$\boxed{30 \text{ ATP}}$

IfAS

Harshada Sharma

Earned: ₹25,500

"As is widely recognized, IFAS stands as India's leading institute for CSIR NET examination preparation. My Personal philosophy has always revolved around helping others and I've had the pleasure of introducing the IFAS Online Course to 12 of my classmates and juniors, which in turn allowed me to earn 25,500 rupees. You too can join in and refer your friends, allowing you to earn a 5% rewards based on the fees each of your referred friends Pay"

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Step – 01 Invite Friends

Share your referral code or Link with your friends.

Step – 02 Earn Cashback

On Every Purchase made using your referral code, you earn 5% instant cashback in your bank account.

Step – 03 Refer More, Earn More

Keep referring as many friends as you can and keep **Earning** cash as much as you can

Friendship is Sharing, Caring, Giving & Helping Each Other...!